

## **REMARKS**

### **Amendments to the claims**

The Applicant has amended claim 1 in order to clarify that in step (a), the aqueous slurry of plant material contains a mixture of neutral sugars which comprise monosaccharides, disaccharides and trisaccharides. Furthermore, step (c) of claim 1 has been clarified to show that the mixture of neutral sugars that are separated along with the neutral fractions in step (c) comprise monosaccharides, disaccharides and trisaccharides.

Support for the amendment can be found at page 2 of the description which describes the neutral sugars in the slurry. A person of ordinary skill in the art would know that a free neutral sugar in solution is defined as a compound which could be grouped as a monosaccharide, a disaccharide or a trisaccharide.

As such, claim 1 has been amended to further clarify the invention and no new matter has been added by way of the amendments to the claim.

### **35 U.S.C. §103**

The objective of the Applicant's invention is to isolate inositol from plant material. Separating inositol from plant material is difficult since inositol is a neutral sugar that is very similar in molecular size and charge characteristics to other sugars such as glucose, fructose and sucrose that are present at a high concentration in a slurry of plant materials. The core of the invention is to utilize a method for the partial hydrolysis of the phytate in the plant material to charge inositol phosphate intermediates, separate these intermediates from the "free" neutral sugars in solution which comprise monosaccharides, disaccharides and trisaccharides, and then complete the full hydrolysis of the intermediates, and then separate the neutral inositol from charged ions and compounds.

Claims 1-20 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Sirén (Siren (US 4,777,134 – hereinafter Sirén), Sirén (US 4,797,390 – hereinafter Sirén 2) and Vanderbeke et al (US 5,554,399).

Example 12 of Sirén teaches the hydrolysis of sodium phytate with wheat bran and the fractionation of a mixture of inositolphosphates. The Example uses a starting material that is purified sodium phytate (sourced from Sigma Chemical). Given the source is purified, the starting material does not contain any of the neutral sugars found in the starting material of the present invention, such as monosaccharides, disaccharides and trisaccharides.

In the first step of Example 12, the sodium phytate is dissolved in the sodium acetate buffer at pH 5.0. In a next step the temperature is increased and the wheat bran is added to the slurry for its phytase activity. The Examiner argues that wheat bran is a plant material containing a mixture of neutral sugars with a phytase enzyme and thus comprises an aqueous slurry thereof.

The Applicant respectfully disagrees that wheat bran contains a mixture of neutral sugars comprising monosaccharides, disaccharides or trisaccharides. A person of ordinary skill in the art would know that wheat bran consists primarily of fiber. Wheat bran contains almost zero neutral sugars and thus by adding wheat bran as the source of enzyme to the sodium phytate, one is not adding any neutral sugars comprising monosaccharides, disaccharides or trisaccharides to the slurry.

The Examiner has cited four publications, namely Chen, Nyman et al, Anderson et al and Theander et al. on pages 7 and 8 of her Report in order to assert that wheat bran contains neutral sugars.

Applicant respectfully disagrees with the Examiner's interpretation of these references and their application to the present invention.

The neutral sugars referred to in the cited references do not exist in the native form of wheat bran. Rather, these sugars are the monomeric components of fiber molecules that compromise the bulk composition of wheat bran. The fiber molecules can be broken down to free neutral sugars only under extreme conditions of sulfuric acid exposure in the laboratory. As such the neutral sugars referred to in the references are in fact components of fiber molecules and distinct from the "free" neutral sugars namely monosaccharides, disaccharides and trisaccharides as found in the plant slurry in steps (a) and (c) of claim 1 of the present invention.

Wheat bran is well established to be composed primarily of fiber which consists of very large macromolecules that can be classified as follows: (a) beta glucans which are very long strands of beta 1-6 linked glucose molecules. There is considerable hydrogen bonding between the strands to result in a rigid fibrous structure and (b) arabinoxylans which are strands of beta 1-6 and beta 1-4 linked xylose and arabinose. These are also highly rigid water insoluble fibrous structures.

Turning to the references cited by the Examiner, for instance, the Chen et al reference at paragraph 3, page 712 describes the methodology used to determine the carbohydrate content of wheat bran. The paragraph refers to an earlier paper by Theander and Westerlund, which is enclosed herewith. As described in the Theander and Westerlund paper, the fiber is subjected to extreme conditions of sulfuric acid (12M) to hydrolyze the fiber macromolecules to individual neutral sugar monomers. The sugars are then measured and the neutral sugar content of the wheat bran is reported.

As described above, the fiber in wheat bran is predominantly highly insoluble but there is a small amount of beta glucans (soluble strands of beta linked glucose molecules). Again this soluble fiber is not in the form of free neutral sugars. Severe acid hydrolysis

is required to break down the fiber for the assay. These conditions would completely destroy any enzyme in the mixture such as phytase.

In the Theander reference at p. 103, Table 1 shows the relative composition of neutral sugars in wheat bran only after acid hydrolysis. Anderson measures the neutral sugar fraction of the cellulose extract of corn bran and compares it to wheat bran he measured in a previous publication, presumably extracted in the same manner as described at page 760 of this reference, namely hydrolyzed with sulfuric acid. Nyman does nothing more than teach the composition and faecal recovery of dietary fibre in wheat bran. The methodology used to measure the monomeric composition of neutral sugars in the fibre residues and in faeces is described at page 489 of Nyman and also involves hydrolysis using  $H_2SO_4$ .

Therefore, as can be seen from the references cited by the Examiner, the level of free neutral sugars in other words in the form of monosaccharides, disaccharides and trisaccharides in wheat bran is negligible and the addition of wheat bran in Example 12 of Siren would not add any neutral sugars to the slurry as any neutral sugar present is bound to the fiber in the wheat bran and can only be liberated after severe acid hydrolysis.

In yet a further step in Example 12, after partial hydrolysis, the resulting supernatant is passed through a column and eluted with increasing concentrations of HCl in order to displace the inositol phosphates. Eluted fractions are then hydrolyzed for the purpose of identifying the various inositol phosphates. However, the mixture that is passed through the column is simply a mixture of inositol phosphates without the neutral sugars. As such, Sirén is merely measuring the various forms of inositol phosphates in order to quantify them and detect  $IP_3$ .

When contrasted with claim 1 of the present invention, step (a) is not taught since there is no treatment of an aqueous slurry of plant material containing a mixture of neutral sugars. The wheat bran is not the starting material that is the subject of the "treatment"

or partial hydrolysis as per claim 1(a). Rather the starting material is sodium phytate which is not a plant material that contains any neutral sugars comprising monosaccharides, disaccharides, or trisaccharides. Furthermore, the addition of wheat bran does not add any neutral sugars to the slurry, as described above.

Step (c) is not present since Example 12 does not separate the water soluble fraction into a first ionic fraction from another neutral fraction which contains neutral sugars comprising monosaccharides, disaccharides, or trisaccharides. Furthermore, step (e) of claim 1 is not taught in Sirén since the step of isolating the inositol from the other charged components is not disclosed.

The Examiner has further cited Sirén 2 at page 4 of her Report. However, column 4, lines 25-38 of Siren 2 referenced by the Examiner merely teaches adding phytase enzyme where the starting material has too low an enzymatic activity to break down the higher inositol phosphates to IP3. Siren 2 mentions ion exchange only in the context of isolating the various inositol triphosphate isomers and not for separating negatively charged inositol phosphates from the other neutral sugars comprising monosaccharides, disaccharides and trisaccharides. Siren 2 does not teach using hydrolysis to manipulate the charge characteristics of the mixture or how to isolate neutral inositol from other neutral sugars in solution such as fructose, glucose and sucrose that are present in the slurry of plant material. Sirén 2 does not teach the present invention, and also does not teach steps (c) and (e) taught by claim 1 of the present invention that are missing from Sirén. Applicant submits that using the phytase enzyme that is normally present in all inositol phosphate containing plants is not always feasible, especially in cases where the starting material has little to no naturally occurring phytase activity, and is further not relevant to a finding of obviousness.

The elements of claim 1 and dependent claims 2-20 are not taught or disclosed by Sirén or Sirén 2 individually, nor by the combination of Sirén and Sirén 2.

The Examiner has further cited Vanderbeke at page 5 of her Report against the present

invention. However, Vanderbeke merely teaches that full hydrolysis is possible with an optimized enzyme composition that displays a higher synergistic phytate hydrolyzing activity at a pH from 2.5 to 5.0 and an acid phosphatase having phytate hydrolyzing activity at a pH of 2.5. Vanderbeke in and of itself does not teach the steps disclosed in claim 1 of the present invention nor what is missing from Sirén and Sirén 2.

In view of the foregoing, Applicant respectfully submits that all pending claims are clearly and patentably distinguished over the references cited by the Examiner and, as such, are in condition for allowance.

Respectfully submitted,

A handwritten signature in black ink, appearing to read 'D. Maravei', is written over a horizontal line.

Daphne L. Maravei  
Patent Agent for Applicant  
Reg. No. 53,881

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BLAKE, CASSELS & GRAYDON LLP  
20<sup>th</sup> Floor  
45 O'Connor Street  
Ottawa, Ontario  
K1P 1A4

Tel: 613-788-2244  
Fax: 613-788-2247